Cancer Incidence among Pesticide Applicators Exposed to Alachlor in the Agricultural Health Study

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The authors evaluated the incidence of cancer among pesticide applicators with exposure to alachlor in the Agricultural Health Study, a prospective cohort study of licensed pesticide applicators in Iowa and North Carolina. A total of 49,980 pesticide applicators are included in this analysis; 26,510 applicators (53%) reported use of alachlor on the enrollment questionnaire. Detailed pesticide exposure and other information were obtained from a self-administered questionnaire completed at the time of enrollment (1993–1997). Poisson regression analysis was used to evaluate the exposure-response relations between alachlor and cancer incidence controlled for the effects of potential confounding factors. A total of 1,466 incident malignant neoplasms were diagnosed during the study period, 1993–2000. Among alachlor-exposed applicators, the authors found a significant increasing trend for incidence of all lymphohematopoietic cancers associated with lifetime exposure-days (p for trend = 0.02) and intensity-weighted exposure-days (p for trend = 0.03) to alachlor. The risks of leukemia (rate ratio = 2.83, 95% confidence interval: 0.74, 10.9) and multiple myeloma (rate ratio = 5.66, 95% confidence interval: 0.70, 45.7) were increased among applicators in the highest alachlor exposure category. Our findings suggest a possible association between alachlor application and incidence of lymphohematopoietic cancers among applicators in the Agricultural Health Study.

agriculture; cohort studies; herbicides; leukemia; multiple myeloma; neoplasms; occupational exposure; pesticides

Abbreviations: CI, confidence interval; OR, odds ratio; SIR, standardized incidence ratio.

Alachlor (2-chloro-2′,6′-diethyl-N-(methoxymethyl)acetanilide) is a pre- and early post-emergent herbicide used mainly in the production of corn, soybeans, and peanuts (1). Alachlor has been marketed since 1969 under the trade name Lasso (Monsanto Company, St. Louis, Missouri). According to US Environmental Protection Agency estimates, alachlor had an annual usage of 7–10 million pounds (1 pound = 0.45 kg) in 1999, making it one of the most widely used herbicides in the United States (2).

Alachlor produced thyroid (3), nasal (4), and stomach cancers (5) in rats presumedly by a nongenotoxic, threshold-sensitive process (6). In 1985, the US Environmental Protection Agency categorized alachlor as a probable human carcinogen (7). There is little epidemiologic information on cancer in alachlor-exposed populations. Two retrospective cohort studies showed elevated risks for colorectal cancer and leukemia among alachlor-manufacturing workers (8, 9). However, interpretation of these findings is difficult because of the small number of observed cancer cases ($n = 23$ (8) and $n = 18$ (9)) and the lack of control for potentially confounding exposures.

The Agricultural Health Study cohort (10) will be used to comprehensively examine the hypothesized link between a wide variety of occupational exposures among farmers and commercial applicators and the risk of cancer and other chronic diseases. Our overall approach is to evaluate risk factors for specific diseases of interest once sufficient numbers of exposed cases have been observed in the cohort (e.g., prostate cancer (11)) and also to evaluate cancer risks among selected exposure groups of a priori interest. For the latter, we first focus on major-use pesticides of biologic interest. In this context, we have chosen to examine the cancer experience of alachlor applicators because alachlor is widely used in US agriculture and it has animal bioassay data, which suggest that it may be a human carcinogen.

MATERIALS AND METHODS

Cohort enrollment and follow-up

The Agricultural Health Study is a prospective cohort study composed of 57,311 applicators licensed to apply restricted use pesticides and the 32,347 spouses of private applicators from Iowa and North Carolina (10). Recruitment of applicators began in December 1993 and continued until December 1997. Cohort members were matched to cancer registry files in Iowa and North Carolina for case identification and to the state death registries and to the National Death Index to ascertain vital status. Incident cancers were identified from the date of enrollment (i.e., 1993–1997) through December 31, 2000, and coded according to the *International Classification of Diseases*, Ninth Revision (12). Cohort members alive but no longer residing in Iowa or North Carolina ($n = 857$) were identified through the current address records of the Internal Revenue Service, motor vehicle registration offices, and pesticide license registries of the state agriculture departments, and they were censored in the year they left the state. The average number of years of follow-up is 5.5 years.

Exposure assessment

A self-administered enrollment questionnaire collected comprehensive exposure data on 22 pesticides and ever/never use information for 28 more pesticides, as well as information on use of personal protective equipment, pesticide application methods, pesticide mixing, equipment repair, smoking, alcohol consumption, cancer history of first-degree relatives, and basic demographic data. (The questionnaire may be found at http://www.aghealth.org). Questionnaire data from the enrollment and measurement data from the published pesticide exposure literature were used to calculate the estimated intensity of exposure to individual pesticides using the formula: intensity level = (mixing status + application method + equipment repair status) × personal protective equipment use (13). We constructed two lifetime alachlor exposure variables, each categorized into quartiles for this analysis: 1) lifetime exposure-days based on the number of years applied and the frequency of application using the midpoints of the questionnaire category (i.e., years of use × days per year: <19.9, 20.0–56.0, 56.1–116.0,

≥116.1) and 2) intensity-weighted exposure-days multiplying lifetime exposure-days by exposure intensity level (i.e., years of use × days per year × intensity level: <101.9, 102.0–253.1, 253.2–710.4, ≥710.5).

Data analysis

Prevalent cancer cases ($n = 1,064$) and applicators who did not provide any information on alachlor use ($n = 6,267$) were excluded from this analysis, leaving 26,510 exposed and 23,470 nonexposed applicators. Those excluded were mainly from North Carolina (69 percent) and were likely to have missing data for other variables.

A standardized incidence ratio for all cancers was calculated as the ratio of observed to expected number of cancer cases using standard methods (14, 15). Expected numbers for the standardized incidence ratio were estimated from 5-year age and calendar-time, race-specific cancer incidence rates from the population-based cancer registries in Iowa and North Carolina. We also conducted site-specific analysis for cancers with five or more exposed cases.

Poisson regression analysis using the Stata program (version 7.0) (16) was conducted while controlling for the effect of potential confounding factors to examine internal exposure-response relations. Rate ratios derived from the analysis were adjusted for age at enrollment (<40, 40–49, 50–59, ≥60 years), sex, education (high school graduate or less, greater than high school), smoking (by pack-years: never/low/high), alcohol drinking during the past 12 months (yes/no), family history of cancer in first-degree relatives (yes/no), state (Iowa/North Carolina), and enrollment year. The median value of pack-years among smokers (12 pack-years) was used to classify the "low" and "high" categories of smokers. The reference group for each rate ratio was the lowest level of lifetime exposure-days or intensity-weighted exposure-days; applicators who reported not using alachlor were excluded from the Poisson regression analyses. Since there was potential confounding from other pesticide exposures, we also adjusted rate ratios for the five most highly correlated pesticides (atrazine, cyanazine, metolachlor, trifluralin, 2,4-dichlorophenoxyacetic acid (2,4-D)), with intensity-weighted exposure-days of alachlor ($r \geq 0.4$). The exposure levels of these five pesticides were categorized as never, low, and high. The low group and the high group of each pesticide were classified by the median intensity-weighted exposure-days of each pesticide. Tests for linear trend were performed to assess exposure-response patterns in each cancer outcome using the method described by Breslow and Day (17). All significance tests were two sided.

RESULTS

Table 1 shows selected characteristics of applicators by alachlor exposure. The majority of the cohort are male private applicators. Among subjects with complete exposure information, 25,532 applicators have used alachlor and have information on lifetime alachlor exposure-days. This group was divided into two parts, that is, the lowest exposed quartile ($n = 5,539$) and the three remaining exposed quartiles ($n = 19,993$). The group with no alachlor use consisted of

TABLE 1. Selected characteristics of applicators, by alachlor exposure, based on 1993–1997 enrollment data in the Agricultural Health Study

Characteristics	The lowest exposed* (n = 5,539)		Other exposed† (n = 19,993)		Nonexposed (n = 23,470)	
	No.	%	No.	%	No.	%
Age (years)						
<40	1,647	29.7	5,709	28.6	9,168	39.1
40–49	1,650	29.8	6,341	31.7	6,062	25.8
50–59	1,210	21.9	4,478	22.4	4,255	18.1
≥60	1,032	18.6	3,465	17.3	3,984	17.0
Gender						
Male	5,450	98.4	19,866	99.4	22,432	95.6
Female	89	1.6	127	0.6	1,038	4.4
State of residence						
Iowa	4,140	74.7	14,387	72.0	15,138	64.5
North Carolina	1,399	25.3	5,606	28.0	8,332	35.5
Applicator type‡						
Private	5,304	95.8	18,241	91.2	20,893	89.0
Commercial	235	4.2	1,752	8.8	2,577	11.0
Smoking history						
Never	3,045	56.5	10,574	54.4	12,678	55.7
Low (<12 pack-years)	1,239	23.0	4,339	22.3	5,117	22.5
High (≥12 pack-years)	1,102	20.5	4,542	23.3	4,950	21.8
Alcohol drinking history§						
No	1,632	29.7	5,546	28.0	7,699	33.4
Yes	3,855	70.3	14,262	72.0	15,371	66.6
Education						
High school or less	2,859	52.8	10,842	55.3	12,827	56.0
Greater than high school	2,555	47.2	8,756	44.7	10,087	44.0
Family history of cancer						
No	2,950	56.1	10,751	56.8	14,005	63.1
Yes	2,305	43.9	8,184	43.2	8,190	36.9
Corn production						
No	1,117	20.2	3,623	18.1	9,504	40.5
Yes	4,422	79.8	16,370	81.9	13,966	59.5
Five pesticides most highly correlated with alachlor						
Atrazine	4,390	80.1	17,647	89.1	11,382	49.2
Cyanazine	2,818	51.3	11,514	58.0	5,314	22.8
Metolachlor	2,975	54.3	12,397	62.5	6,610	28.4
Trifluralin	3,353	61.2	12,686	64.1	8,356	36.1
2,4-D¶	4,569	83.7	17,273	87.5	13,993	60.9

* First quartile of lifetime exposure-days (years of use × days per year).

† Second, third, and fourth quartiles of lifetime exposure-days (years of use × days per year).

‡ The term "private applicators" refers primarily to individual farmers, and "commercial" refers to professional pesticide applicators.

§ Based on the question, "Did you ever drink any kind of alcoholic beverage during the past 12 months?".

¶ 2,4-D, 2,4-dichlorophenoxyacetic acid.

23,470 applicators. The lowest exposed quartile is observed to be more similar to the remaining three exposed quartiles than to the nonexposed group of applicators on a number of important variables. These include age, sex, state of residence, family history of cancer, type of farm (corn production), and the number of different pesticides used in a

TABLE 2. Standardized incidence ratios and rate ratios for selected cancers* by alachlor exposure status of the Agricultural Health Study applicators, 1993–2000

Cause of cancer (ICD-9† classification)	Exposed			Nonexposed			Exposed/nonexposed	
	Observed (no.)	SIR†	95% CI†	Observed (no.)	SIR	95% CI	RR†,‡	95% CI
All malignant neoplasms (codes 140–208)	805	0.80	0.74, 0.85	661	0.81	0.75, 0.87	1.01	0.88, 1.15
Buccal cavity, pharynx (codes 140–149)	22	0.60	0.37, 0.91	23	0.81	0.52, 1.22	0.71	0.34, 1.49
Esophagus (code 150)	12	0.79	0.41, 1.39	4	0.35	0.09, 0.89	1.77	0.46, 6.74
Stomach (code 151)	8	0.51	0.22, 1.01	10	0.82	0.39, 1.50	0.37	0.12, 1.15
Colorectal (codes 153 and 154)	87	0.72	0.58, 0.89	72	0.76	0.59, 0.95	0.82	0.55, 1.21
Colon (code 153)	61	0.76	0.58, 0.98	49	0.77	0.57, 1.01	0.86	0.54, 1.37
Rectum (code 154)	26	0.64	0.42, 0.94	23	0.74	0.47, 1.11	0.74	0.36, 1.51
Liver (codes 155 and 156)	7	1.09	0.44, 2.25	5	0.96	0.31, 2.25	1.36	0.32, 5.78
Pancreas (code 157)	13	0.61	0.33, 1.05	14	0.83	0.45, 1.39	0.49	0.20, 1.24
Larynx (code 161)	7	0.36	0.14, 0.74	4	0.26	0.07, 0.67	3.33	0.62, 17.78
Lung (code 162)	83	0.44	0.35, 0.54	63	0.41	0.31, 0.52	1.11	0.70, 1.77
Melanoma (code 172)	38	1.00	0.70, 1.37	20	0.64	0.39, 0.99	1.59	0.83, 3.05
Prostate (code 185)	325	1.16	1.04, 1.30	246	1.13	0.99, 1.28	1.13	0.91, 1.41
Testis (code 186)	8	0.89	0.38, 1.75	11	1.23	0.61, 2.21	0.75	0.25, 2.23
Bladder (code 188)	30	0.49	0.33, 0.71	25	0.51	0.33, 0.75	1.07	0.54, 2.12
Kidney (code 189)	23	0.74	0.47, 1.11	24	0.99	0.63, 1.47	0.63	0.28, 1.42
Brain (codes 191 and 192)	13	0.84	0.44, 1.43	11	0.87	0.43, 1.55	0.99	0.34, 2.90
Thyroid (code 193)	10	1.27	0.61, 2.33	6	0.90	0.33, 1.97	1.63	0.42, 6.37
All lymphohematopoietic cancers (codes 200–208)	70	0.85	0.66, 1.07	65	0.98	0.76, 1.25	0.83	0.54, 1.26
Non-Hodgkin's lymphoma (codes 200 and 202)	29	0.73	0.49, 1.05	32	1.00	0.69, 1.41	0.50	0.26, 0.94
Multiple myeloma (code 203)	11	1.04	0.52, 1.87	11	1.30	0.65, 2.33	0.93	0.30, 2.84
Leukemia (codes 204–208)	26	0.94	0.61, 1.38	19	0.88	0.53, 1.38	1.53	0.72, 3.25

* Cancer subtypes with fewer than five exposed cases are not shown.

† ICD-9, *International Classification of Diseases*, Ninth Revision; SIR, standardized incidence ratio; CI, confidence interval; RR, rate ratio.

‡ Rate ratio adjusted for age, sex, alcohol, smoking, education, family history of cancer, enrollment year, state of residence, and the five pesticides most highly correlated with alachlor (atrazine, cyanazine, metolachlor, trifluralin, 2,4-D (2,4-dichlorophenoxyacetic acid)). The reference category was applicators who were not exposed to alachlor.

lifetime—variables that could be surrogates of important occupational and environmental exposures not explicitly identified by our study questionnaire but potential confounders in our analysis.

A total of 805 and 661 incident cancers were observed among alachlor-exposed and alachlor-nonexposed applicators, respectively (table 2). The standardized incidence ratio analysis indicates that both the alachlor-exposed (standardized incidence ratio (SIR) = 0.80, 95 percent confidence interval (CI): 0.74, 0.85) and the alachlor-nonexposed (SIR = 0.81, 95 percent CI: 0.75, 0.87) groups show a significant reduced risk of cancer overall compared with those expected based on incidence rates in Iowa and North Carolina. This is particularly true for smoking-related cancers, for example, lung cancer, for we observed a standardized incidence ratio of 0.44 (95 percent CI: 0.35, 0.54) among those exposed to alachlor and a standardized incidence ratio of 0.41 (95 percent CI: 0.31, 0.52) among those nonexposed. Findings for bladder cancer were similar with a significantly reduced risk among those exposed (SIR = 0.49) and those nonexposed (SIR = 0.51). For colorectal cancer and cancers

not typically associated with smoking, a significantly reduced risk was observed for both the alachlor-exposed group (SIR = 0.72) and the nonexposed group (SIR = 0.76). For prostate cancer, a small but similar excess was seen in both the exposed (SIR = 1.16) and nonexposed (SIR = 1.13) groups. A marginally lower risk for melanoma was observed among the nonexposed alachlor group (SIR = 0.64) but not among those exposed to alachlor (SIR = 1.00).

The non-Hodgkin's lymphoma risk (rate ratio = 0.50) was significantly lower than expected when we directly compared those exposed to alachlor versus those nonexposed by the mean of relative risks controlled for age, sex, alcohol, smoking, education, family history of cancer, enrollment year, state of residence, and the five pesticides most highly correlated with alachlor (table 2). All other relative risks are in the expected range. The results from state-specific analyses were similar (not shown).

The rate ratios for selected cancers, including those of a priori interest (nasal cavity, stomach, thyroid), are reported in table 3 by quartiles of lifetime exposure-days and intensity-weighted alachlor exposure-days. For all cancers

TABLE 3. Rate ratios for selected cancers by lifetime exposure-days and intensity-weighted exposure-days to alachlor among Agricultural Health Study applicators, 1993–2000

	All neoplasms			All lymphohematopoietic cancers			Leukemia			Multiple myeloma			Non-Hodgkin's lymphoma		
	No. of cases	RR*,†	95% CI*	No. of cases	RR	95% CI	No. of cases	RR	95% CI	No. of cases	RR	95% CI	No. of cases	RR	95% CI
Lifetime alachlor exposure-days‡															
0.1–19.9§	148	1.0		14	1.0		6	1.0		3	1.0		5	1.0	
20.0–56.0	221	1.22	0.96, 1.55	12	0.67	0.27, 1.66	6	0.76	0.19, 3.07	1	0.48	0.04, 5.47	4	0.55	0.12, 2.48
56.1–116.0	180	1.34	1.04, 1.73	16	1.59	0.70, 3.63	4	1.16	0.28, 4.82	2	1.53	0.20, 11.8	8	1.50	0.42, 5.40
≥116.1	206	1.27	0.97, 1.65	26	2.04	0.89, 4.65	10	3.01	0.82, 11.0	5	2.99	0.47, 19.0	10	1.14	0.30, 4.36
Trend¶		0.08			0.02			0.05			0.14			0.53	
Intensity-weighted alachlor exposure-days#															
0.1–101.9§	189	1.0		15	1.0		7	1.0		3	1.0		5	1.0	
102.0–253.1	188	1.37	1.09, 1.74	12	0.99	0.40, 2.44	7	0.99	0.27, 3.58	2	2.22	0.29, 16.9	3	0.62	0.11, 3.44
253.2–710.4	188	1.42	1.11, 1.82	18	2.14	0.95, 4.83	3	0.85	0.19, 3.73	1	1.12	0.09, 14.4	10	2.40	0.65, 8.82
≥710.5	189	1.11	0.84, 1.46	23	2.42	1.00, 5.89	9	2.83	0.74, 10.9	5	5.66	0.70, 45.7	9	1.40	0.32, 6.11
Trend		0.39			0.03			0.17			0.13			0.42	

	Bladder cancer			Colon cancer			Rectal cancer			Stomach cancer			Thyroid cancer		
	No. of cases	RR	95% CI	No. of cases	RR	95% CI	No. of cases	RR	95% CI	No. of cases	RR	95% CI	No. of cases	RR	95% CI
Lifetime alachlor exposure-days															
0.1–19.9§	4	1.0		11	1.0		6	1.0		3	1.0		3	1.0	
20.0–56.0	7	1.25	0.30, 5.27	17	1.68	0.69, 4.14	3	0.32	0.06, 1.78	1	0.27	0.02, 3.35	3	1.17	0.10, 13.2
56.1–116.0	5	1.72	0.40, 7.45	18	2.00	0.79, 5.09	8	1.68	0.48, 5.83	1	0.25	0.02, 3.47	2	1.53	0.13, 17.8
≥116.1	13	3.65	0.92, 14.5	13	0.62	0.20, 1.99	7	0.71	0.16, 3.11	2	0.49	0.05, 4.78	2	1.27	0.10, 16.4
Trend		0.03			0.53			0.77			0.63			0.82	
Intensity-weighted alachlor exposure-days															
0.1–101.9§	4	1.0		14	1.0		6	1.0		4	1.0		3	1.0	
102.0–253.1	8	3.11	0.79, 12.3	12	1.36	0.56, 3.34	7	1.68	0.46, 6.17	0			2	2.17	0.19, 24.9
253.2–710.4	10	3.64	0.89, 14.8	16	1.85	0.76, 4.50	3	0.75	0.15, 3.73	1	0.20	0.02, 2.52	2	0.93	0.05, 16.5
≥710.5	7	2.03	0.41, 10.0	17	1.14	0.40, 3.23	8	1.07	0.23, 5.07	2	0.22	0.02, 2.26	3	2.89	0.22, 38.7
Trend		0.44			0.64			0.83			0.31			0.54	

* RR, rate ratio; CI, confidence interval.

† Rate ratio adjusted for age, sex, alcohol, smoking, education, family history of cancer, enrollment year, state of residence, and the five pesticides most highly correlated with alachlor (atrazine, cyanazine, metolachlor, trifluralin, 2,4-D (2,4-dichlorophenoxyacetic acid)).

‡ Lifetime exposure-days = years of use × days per year.

§ Reference group.

¶ *p* value for trend test.

\# Intensity-weighted exposure-days = years of use × days per year × intensity index.

combined, there was no trend of increasing risk with increasing exposure. A significant exposure-response trend was observed for all lymphohematopoietic cancers combined using either the lifetime exposure-days or intensity-weighted exposure-days, rising to an over twofold increased risk in the highest category. The risks of leukemia and multiple myeloma were markedly increased in the highest exposure category, although they had wide confi-

dence intervals. These results were not changed when we added "total years of pesticide application" to the multivariate analysis as a surrogate measure of other potential farming exposures (data not shown). A significant trend for bladder cancer was observed for the lifetime exposure-days but not the intensity-weighted exposure-days. Similar analyses for other cancers did not suggest associations. An analysis that was restricted to private applicators living in Iowa

yielded results similar to those reported in table 3. The results for North Carolina applicators were unstable as a result of smaller numbers of cases.

DISCUSSION

We found significant positive exposure-response trends among pesticide applicators exposed to alachlor for all lymphohematopoietic cancers combined, using two exposure measures (i.e., lifetime exposure-days and intensity-weighted exposure-days). Among the lymphohematopoietic cancers, leukemia and multiple myeloma showed this pattern independently, but the numbers were small. The findings were similar when we repeated the exposure-response analyses restricting the study population to Iowa or to private applicators.

Elevated risks for all lymphohematopoietic cancers (SIR = 3.6, 95 percent CI: 1.2, 8.5) and chronic myeloid leukemia (SIR = 25.0, 95 percent CI: 3.0, 90.3) have been reported for alachlor-manufacturing workers (9). A subsequent analysis of these manufacturing workers also showed an increased risk for chronic myeloid leukemia (8). However, population-based case-control studies have shown no significant association between alachlor exposure and leukemia (odds ratio (OR) = 1.0, 95 percent CI: 0.7, 1.5) (18), non-Hodgkin's lymphoma (OR = 1.2, 95 percent CI: 0.8, 1.7) (19), or multiple myeloma (OR = 0.9, 95 percent CI: 0.5, 1.7) (20). As with many population-based case-control studies, these were limited by the relatively small numbers of exposed cases and the fact that exposure assessment was conducted after disease diagnosis.

There is some evidence of a genotoxic effect of alachlor in experimental systems. In mammals, alachlor induced chromosomal aberrations in bone marrow cells of Wistar rats treated in vivo (21) and in cultured Chinese hamster ovary cells (22). Increased thyroid, stomach, and nasal tumors were observed in Long-Evans and Sprague-Dawley rats associated with alachlor exposure (3–6). Alachlor also has been found to cause chromosomal damage in human lymphocytes (21, 23) exposed in vitro.

Our standardized incidence ratio analysis of those exposed and those nonexposed to alachlor indicates that the two groups are both at significantly reduced risk of cancer overall and particularly to smoking-related cancers of the lung and bladder. A similar reduced risk is observed among both groups for colorectal cancer, which may in part be due to the more physically active work of farmers and commercial pesticide applicators compared with other residents of Iowa and North Carolina. The fact that melanoma is marginally lower than expected among the nonexposed group and not among the exposed group may indicate that nonexposed cohort members are less frequently exposed to excess sunlight exposure. Although comparing all those ever exposed with those never exposed to alachlor in a standardized incidence ratio analysis can establish whether the two groups have similar cancer risk profiles, standardized incidence ratios are a relatively insensitive indicator of occupational risk because they cannot adequately control for significant confounding factors and have well-established "healthy worker" problems. The significantly lower risk of

non-Hodgkin's lymphoma observed among those exposed to alachlor compared with those not exposed while controlling for age, sex, alcohol, smoking, education, family history of cancer, enrollment year, state of residence, and the five most highly correlated pesticides with alachlor is unexpected. This "protective effect" of alachlor is not observed in the subsequent dose-response analysis described in table 3, and it is likely therefore to be a statistical artifact due to either chance or residual confounding resulting from exposure not explicitly identified by our questionnaire.

Although a significant 1.3-fold risk for all cancers combined was found among applicators in the second highest category of lifetime exposure to alachlor, the lack of a monotonic exposure-response relation suggests that this increase may be due to chance. Moreover, we did not find increased cancer risks for several sites of a priori interest that were positive in animal bioassays including the nose, stomach, and thyroid gland. Since the number of observed cases at these sites was small during our short follow-up study, continued follow-up is warranted. Although the use of positive animal bioassays to indicate the potentially important biologic activity of a chemical is standard practice, extrapolating organ site-specific experimental results to humans is problematic because of differences in human metabolism, physiology, and environmental conditions (24).

A previous cohort study (9) reported increased risk (SIR) for colorectal cancer among alachlor-manufacturing workers. However, Acquavella et al. (8) suggested that the association between alachlor and colorectal cancer was noncausal because they did not observe any cases in a highly exposed department and because the colon plays only a minor role in alachlor metabolism and excretion. We saw no excess for colorectal cancer in the standardized incidence ratio analysis nor any trend by level of exposure.

Our observation of a slightly increased standardized incidence ratio for prostate cancer among both alachlor-exposed and alachlor-nonexposed applicators is consistent with a previous report from this study and with results from other studies of farmers (25–30). Recently, Alavanja et al. (11) reported that chlorinated pesticides and methyl bromide (but not alachlor) were significantly associated with an excess risk of prostate cancer in this cohort. We did not see any association between alachlor and prostate cancer in the Poisson analyses.

An increasing trend for bladder cancer associated with lifetime exposure-days was observed. The lack of a corresponding increase with the intensity-weighted exposure-days is difficult to explain but argues against a causal relation.

Overall, 17 percent of applicators in this cohort are current smokers, which rate is lower than for the United States as a whole (28 percent for males and 23 percent for females) (31). The observed deficits for smoking-related cancers, such as cancers of the lung, bladder, larynx, and buccal cavity, compared with rates for the general population in Iowa and North Carolina are consistent with the low prevalence of smoking in the Agricultural Health Study cohort. Our exposure-response analyses are adjusted for tobacco use and other potentially important confounders.

Although the period of cohort follow-up is still relatively short (5.5 years on average), this study has several important strengths. The Agricultural Health Study is the largest study of pesticide applicators exposed to alachlor conducted to date. All exposure information was collected prior to the diagnosis of cancer, which obviates case-recall bias. This study included comprehensive questionnaire data that were used to quantitatively estimate alachlor exposure levels and to control for potential confounding (10, 13).

A limitation of this study and almost all studies of pesticide users is that persons who apply pesticides are seldom exposed to just a single agent and that potential confounding is, therefore, possible. Coble et al. (32) evaluated the relations among different agricultural exposures and found that substantial bias due to confounding from exposure to multiple agents was unlikely in this cohort. However, the significant difference observed between alachlor users and nonusers (table 1) for age, gender, state of residence, applicator type, family history of cancer, corn production, and other coexposure to pesticides suggests the possibility that unrecognized residual confounding may bias the dose-response relation in our analysis. To mitigate the possibility of residual confounding, we chose the lowest alachlor exposure group as the referent in our rate ratio analysis rather than the nonexposed group, and we adjusted the lymphohematopoietic risk estimates by including the five pesticides most highly correlated with alachlor in our models.

A total of 857 (less than 1 percent) cohort members left the states of Iowa and North Carolina during the period of the study from 1993 through 2000, and any cancers resulting from this group are lost to the state cancer registry. This small portion of the total cohort is younger and more educated, smokes less, and has a slightly lower frequency of family history of cancer than the total cohort (data not shown) and is therefore likely to generate proportionally fewer cancers than the rest of the cohort. To assess the magnitude of the potential bias caused by having this group of low-cancer-risk study subject leave the cohort, we recalculated our risk estimated by adding all the lost person-years generated by this group to the denominator and assumed no cancer cases in the numerator. We observed only minimal changes in our risk estimates that did not affect our conclusion.

Additionally, the formulation and use of alachlor may have changed over the years. These factors create a potential for some exposure misclassification, particularly in studies where exposure is based on subject recall. Subjects in this study were asked to recall pesticide use over their lifetime. Recall of pesticide use by the Agricultural Health Study cohort has been shown to be similarly reliable to that for other factors routinely evaluated by questionnaire in epidemiology studies, such as smoking and alcohol use, and to be better than others, such as consumption of fruits and vegetables and physical activity (33). Hoppin et al. (34) also demonstrated that participants in our cohort provided plausible information regarding the duration of use of specific pesticides.

Our study had relatively low statistical power to detect excess risks for less common cancers. The statistical power to detect a 1.5-fold increase in the incidence of cancers of a priori interest varied by site, from 75 percent for lymphohematopoietic tissue to 63 percent for colon, 17 percent for stomach, and 12 percent for thyroid cancer.

Although the interpretation of our results is limited by a small number of cases, our findings suggest a possible association between alachlor application and the incidence of lymphohematopoietic cancers—in particular leukemia and multiple myeloma—among applicators in the Agricultural Health Study. Additional follow-up of this cohort will shed further light on the risks for these and other cancers as the number of cancer cases increases over time.

REFERENCES

1. Hayes WJ Jr, Laws ER Jr, eds. Handbook of pesticide toxicology. Vol 3. Classes of pesticides. New York, NY: Academic Press, Inc, 1991:1341–3.
2. Donaldson D, Kiely T, Grube A. Pesticide industry sales and usage: 1998 and 1999 market estimates. Washington, DC: Environmental Protection Agency, 2002. (http://www.epa.gov/oppbead1/pestsales/99pestsales/market_estimates1999.pdf).
3. Wilson AG, Thake DC, Heydens WE, et al. Mode of action of thyroid tumor formation in the male Long-Evans rat administered high doses of alachlor. Fundam Appl Toxicol 1996;33:16–23.
4. Genter MB, Burman DM, Dingeldein MW, et al. Evolution of alachlor-induced nasal neoplasms in the Long-Evans rat. Toxicol Pathol 2000;28:770–81.
5. Thake DC, Iatropoulos MJ, Hard GC, et al. A study of the mechanism of butachlor-associated gastric neoplasms in Sprague-Dawley rats. Exp Toxicol Pathol 1995;47:107–16.
6. Heydens WF, Wilson AG, Kier LD, et al. An evaluation of the carcinogenic potential of the herbicide alachlor to man. Hum Exp Toxicol 1999;18:363–91.
7. Special review of certain pesticide products—alachlor. Springfield, VA: Office of Pesticide Programs, Environmental Protection Agency, 1985. (Document no. PB85-175503).
8. Acquavella JF, Riordan SG, Anne M, et al. Evaluation of mortality and cancer incidence among alachlor manufacturing workers. Environ Health Perspect 1996;104:728–33.
9. Leet T, Acquavella J, Lynch C, et al. Cancer incidence among alachlor manufacturing workers. Am J Ind Med 1996;30:300–6.
10. Alavanja MC, Sandler DP, McMaster SB, et al. The Agricultural Health Study. Environ Health Perspect 1996;104:362–9.
11. Alavanja MC, Samanic C, Dosemeci M, et al. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. Am J Epidemiol 2003;157:800–14.
12. World Health Organization. International classification of diseases. Manual of the international statistical classification of diseases, injuries, and causes of death. Ninth Revision. Geneva, Switzerland: World Health Organization, 1979.
13. Dosemeci M, Alavanja MC, Rowland AS, et al. A quantitative approach for estimating exposure to pesticides in the Agricultural Health Study. Ann Occup Hyg 2002;46:245–60.
14. Lidell F. Simple exact analysis of the standardized mortality ratio. J Epidemiol Community Health 1984;38:85–8.
15. Monson RR. Occupational epidemiology. 2nd ed. Boca Raton, FL: CRC Press, 1990:105–31.
16. StataCorp. Stata reference manual: release 7. College Station, TX: Stata Press, 2001.
17. Breslow NE, Day NE. Statistical methods in cancer research. Vol II. The design and analysis of cohort studies. Lyon, France:

International Agency for Research on Cancer, 1987:91–103. (IARC scientific publication no. 82).

18. Brown LM, Blair A, Gibson R, et al. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res 1990;50:6585–91.

19. Cantor KP, Blair A, Everett G, et al. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res 1992;52:2447–55.

20. Brown LM, Burmeister LF, Everett GD, et al. Pesticide exposures and multiple myeloma in Iowa men. Cancer Causes Control 1993;4:153–6.

21. Georgian L, Moraru I, Draghicescu T, et al. Cytogenetic effects of alachlor and mancozeb. Mutat Res 1983;116:341–8.

22. Lin MF, Wu CL, Wang TC. Pesticide clastogenicity in Chinese hamster ovary cells. Mutat Res 1987;188:241–50.

23. Meisner LF, Belluck DA, Roloff BD. Cytogenetic effects of alachlor and/or atrazine in vivo and in vitro. Environ Mol Mutagen 1992;19:77–82.

24. Madle S, Obe G. Methods for analysis of the mutagenicity of indirect mutagens/carcinogens in eukaryotic cells. Hum Genet 1980;56:7–20.

25. Blair A, Zahm SH. Cancer among farmers. Occup Med 1991;6: 335–54.

26. Blair A, Zahm SH. Agricultural exposures and cancer. Environ Health Perspect 1995;103(suppl 8):205–8.

27. Morrison H, Savitz D, Semenciw R, et al. Farming and prostate cancer mortality. Am J Epidemiol 1993;137:270–80.

28. Aronson KJ, Siemiatycki J, Dewar R, et al. Occupational risk factors for prostate cancer: results from a case-control study in Montreal, Quebec, Canada. Am J Epidemiol 1996;143:363–73.

29. Keller-Byrne JE, Khuder SA, Schaub EA. Meta-analyses of prostate cancer and farming. Am J Ind Med 1997;31:580–6.

30. Acquavella J, Olsen G, Cole P, et al. Cancer among farmers: a meta-analysis. Ann Epidemiol 1998;8:64–74.

31. Centers for Disease Control and Prevention. Smoking and health: United States National Health Interview surveys, selected years 1965–1991. Atlanta, GA: Centers for Disease Control and Prevention, 1995.

32. Coble J, Hoppin JA, Engel L, et al. Prevalence of exposure to solvents, metals, grain dust, and other hazards among farmers in the Agricultural Health Study. J Expo Anal Environ Epidemiol 2002;12:418–26.

33. Blair A, Tarone R, Sandler D, et al. Reliability of reporting on life-style and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. Epidemiology 2002; 13:94–9.

34. Hoppin JA, Yucel F, Dosemeci M, et al. Accuracy of self-reported pesticide use duration information from licensed pesticide applicators in the Agricultural Health Study. J Expo Anal Environ Epidemiol 2002;12:313–18.